



Aalborg Universitet

AALBORG UNIVERSITY
DENMARK

Fibromyalgia

Genetics and epigenetics insights may provide the basis for the development of diagnostic biomarkers

D'Agnelli, Simona; Arendt-Nielsen, Lars; Gerra, Maria C; Zatorri, Katia; Boggiani, Lorenzo; Baciarello, Marco; Bignami, Elena

Published in:
Molecular Pain

DOI (link to publication from Publisher):
[10.1177/1744806918819944](https://doi.org/10.1177/1744806918819944)

Creative Commons License
CC BY-NC 4.0

Publication date:
2019

Document Version
Publisher's PDF, also known as Version of record

[Link to publication from Aalborg University](#)

Citation for published version (APA):

D'Agnelli, S., Arendt-Nielsen, L., Gerra, M. C., Zatorri, K., Boggiani, L., Baciarello, M., & Bignami, E. (2019). Fibromyalgia: Genetics and epigenetics insights may provide the basis for the development of diagnostic biomarkers. *Molecular Pain*, 15, 1-12. [1744806918819944]. <https://doi.org/10.1177/1744806918819944>

General rights


Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal -

Take down policy

If you believe that this document breaches copyright please contact us at vbn@aub.aau.dk providing details, and we will remove access to the work immediately and investigate your claim.

Fibromyalgia: Genetics and epigenetics insights may provide the basis for the development of diagnostic biomarkers

Molecular Pain
Volume 15: 1–12
© The Author(s) 2019
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/1744806918819944
journals.sagepub.com/home/mpx


Simona D'Agnelli¹ , Lars Arendt-Nielsen², Maria C Gerra²,
Katia Zatorri¹, Lorenzo Boggiani³, Marco Baciarello¹, and
Elena Bignami¹

Abstract

Fibromyalgia is a disease characterized by chronic widespread pain with additional symptoms, such as joint stiffness, fatigue, sleep disturbance, cognitive dysfunction, and depression. Currently, fibromyalgia diagnosis is based exclusively on a comprehensive clinical assessment, according to 2016 ACR criteria, but validated biological biomarkers associated with fibromyalgia have not yet been identified. Genome-wide association studies investigated genes potentially involved in fibromyalgia pathogenesis highlighting that genetic factors are possibly responsible for up to 50% of the disease susceptibility. Potential candidate genes found associated to fibromyalgia are *SLC64A4*, *TRPV2*, *MYTIL*, and *NRXN3*. Furthermore, a gene-environmental interaction has been proposed as triggering mechanism, through epigenetic alterations: In particular, fibromyalgia appears to be characterized by a hypomethylated DNA pattern, in genes implicated in stress response, DNA repair, autonomic system response, and subcortical neuronal abnormalities. Differences in the genome-wide expression profile of microRNAs were found among multiple tissues, indicating the involvement of distinct processes in fibromyalgia pathogenesis. Further studies should be dedicated to strength these preliminary findings, in larger multicenter cohorts, to identify reliable directions for biomarker research and clinical practice.

Keywords

Fibromyalgia, genetics, epigenetics, biomarkers, genome-wide association study, DNA methylation, miRNAs

Date Received: 11 September 2018; revised 3 November 2018; accepted: 21 November 2018

Introduction

Fibromyalgia (FM) is a common and complex chronic pain syndrome, affecting 1% to 5% of the population,¹ characterized by chronic widespread pain persisting for more than three months without any obvious organic lesion. Joint stiffness, fatigue, sleep disturbance, cognitive dysfunction, and depression are additional symptoms found associated with FM.^{2,3}

The disease is more common in female than male,⁴ with a ratio of 2:1 similarly to other chronic pain conditions, and it can occur at any age.⁵ Since women show lower pain threshold and more severe symptoms than men,⁶ the majority of researches focused on female subjects. However, the pathogenesis of FM is not fully understood, especially because compared to neuropathic conditions in FM, the source of sensory inputs is

unknown;⁷ some hypothesis on peripheral and central pathophysiological mechanisms have been proposed. Evidence support a central sensitization and a central dysregulation at a spinal and supra-spinal levels in FM patients compared to controls: FM patients showed an exaggerated pain response after sensory stimulation and

¹Anesthesiology, Critical Care and Pain Medicine Division, Department of Medicine and Surgery, University of Parma, Parma, Italy

²Department of Health Science and Technology, Aalborg University, Denmark

³Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parma, Italy

Corresponding Author:

Simona D'Agnelli, Department of Medicine and Surgery, University of Parma, Via Gramsci 14 Road, 43126 Parma, Italy.
Email: simona.dagnelli@studenti.unipr.it



an extended cutaneous silent period;^{8,9} in healthy subjects, the application of an intense painful stimulus produces generalized whole-body analgesia, defined as *conditioned pain modulation*, while it is consistently reduced or even absent in FM subjects;^{10,11} these observations lead to hypothesize a decreased serotonergic and noradrenergic activities.^{12,13} The related neurotransmitters are involved in one of the principal descending monoaminergic pain control pathways¹⁴ and thus play a fundamental role in the mechanism underlying acute and chronic pain.¹⁵ Moreover, the reward/punishment circuit appears to be impaired in FM patients, consistently with the altered dopaminergic/GABAergic neurotransmission.¹⁶ Even functional neuroimaging studies support the altered central neural processing in nociceptive pathways: Following pressure stimuli, a higher activation in brain pain-processing regions was observed in FM subjects compare to controls.¹⁷

The difficulty to identify a specific physiological pathway is also accompanied by difficulties in FM diagnosis, currently only based on a comprehensive clinical assessment; up to 2010, this was principally relying on the 1990 ACR criteria¹⁸ of widespread pain, with at least 3 months consecutive pain and 11 painful “tender points” with digital palpation. Since 2010, new ACR criteria consider other two parameters: The widespread pain index, which locates pain or tenderness in specific body areas, and the symptom severity scale score, which considers both somatic and cognitive symptoms, as trouble thinking or remembering, fatigue, unrefreshed sleep, and depression.¹⁹ Tender points and algometer measurement of pressure pain threshold are still fundamental steps for a comprehensive muscle-skeletal clinical examination

and to exclude other diagnosis linked to widespread pain.¹² In 2016, previous criteria have been reviewed to minimize misclassification of other pain conditions, and FM diagnosis can now be made irrespective on other potential coexisting pathologies, if all the other key symptoms are present.²⁰ Nonetheless, the individual phenotypic variability and concomitant pathologies in the majority of patients lead to non-exhaustive clinical examinations for a precise diagnosis, making tough to define universal criteria for this condition. Furthermore, validated biological biomarkers have not yet been identified; research is thus oriented to discover possible new indicators for an objective diagnosis of affected individuals through the identification of genetic, environmental, and epigenetics factors underlying FM pathophysiology.²¹

Genetic contribution to FM development

Genetic variants and inheritance mechanisms in pain-related genes have been shown to contribute to 50% in the development of chronic pain, as shown by earlier linkage studies, illustrating the correlation between genetic variants and pain response.²² At present, hundreds of pain-regulated genes potentially relevant to pain sensitivity or analgesia have been detected, among which genes encoding for voltage-gated sodium-channels, GTP cyclohydrolase 1, mu-opioid receptors, catechol-O-methyltransferase, and GABAergic pathway proteins.²³

Even if many single nucleotide polymorphisms (SNPs) have been identified as potential candidates specifically associated to FM susceptibility (Table 1), the

Table 1. SNPs related to genes potentially involved in fibromyalgia's pathogenesis.

SNPs	Gene	Clinical relevance
5-HTTLPR ²⁴	SLC6A4	Temporal mandibular joint disorder Depression Psychological disorders
rs4680 ²⁸	COMT	Depression Anxiety Disability
rs1048101 ²⁹	HTR2A	FIQ disability
rs6313 ^{30,31}	HTR2A	Fibromyalgia onset
rs11127292 ³²	MYT1L	Cognitive disability
Intronic CNV ³²	NRXN3	Autism
rs8192619, rs4129256 ³³	TAAR1	Impaired dopamine availability Enhanced pain sensitivity
rs10799897, rs2842003, rs2805050 ³³	RGS4	Alteration in the descending inhibition of pain perception
rs6454674, rs1078602, rs10485171 ³³	CNR1	Migraine Irritable bowel syndrome Post-traumatic stress disorder
rs642544, rs17104711, rs2510177, rs10895837 ³³	GRIA4	Central sensitization

SNP: Single Nucleotide Polymorphism; CNV: copy number variant.

low number of subjects involved did not often allow to confirm them in subsequent meta-analyses.

However, a genome-wide linkage scan study revealed a 13.6-fold increased risk of developing the syndrome in first-degree relatives²⁴ strengthening the genetic hypothesis. The research evidenced a linkage at markers *D17S2196* and *D17S1294* on chromosome 17p11.2–q11.2; two potential FM susceptibility candidate genes map on this region, the serotonin transporter gene (*SLC64A4*), and the transient receptor potential vanilloid channel 2 gene (*TRPV2*).²⁴ *SLC64A4* polymorphisms were already found associated with chronic pain conditions, like temporal mandibular joint disorder;²⁵ in addition, an alteration in serotonin reuptake was associated with high levels of depression and psychological disorders in the same patients.²⁶ Alterations in *TRPV2*, a gene expressed in mechano- and thermo-responsive neurons in the dorsal root and trigeminal ganglia,²⁷ could instead contribute to the impaired pain threshold in FM patients.

Candidate genes-associated studies report a correlation between *Val158Met* variant in *COMT* gene²⁸ and depression, anxiety and disability in FM women, (*1A*)-*AR*-*rs1383914* SNP and FM susceptibility, the (*1A*)-*AR*-*rs1048101* SNP and FIQ disability,²⁹ and *T102C* polymorphism of the *5-HT2A* receptor gene and FM onset.³⁰

In order to clarify the potential association between gene polymorphisms in *5-HTT*, *COMT*, and *5-HT2A* genes and FM susceptibility, Lee et al.³¹ have led a meta-analysis on FM genetic predisposition, highlighting the potential central role of *102T/C* polymorphism in *5-HT2A* receptor; the significant associations of *5-HTTLPR* S/L allele and *COMT* Val158Met with FM were not confirmed.³¹ More investigations need to understand the role of these genes in pain biology and in chronic pain diseases as FM.

Genome-wide association studies have contributed to sustain the possible involvement of central nervous system (CNS) dysfunction in FM. Recently, Docampo et al.³² conducted a genome-wide association studies and copy number variant analyses in 952 FM cases and 644 controls. Their results showed two FM-associated variants, *rs11127292* SNP and an intronic copy number variant, belonging respectively to *MYT1L* (myelin transcription factor 1 like gene), which plays a key role in neuronal differentiation and it is involved in cognitive disability, and to *NRXN3* (neurexin 3 gene), which acts in the nervous system as receptor and cell adhesion molecule, and its genetic variants have been found involved in autism spectrum disorder.³²

However, no SNPs have achieved the genome-wide significant threshold and, therefore, further analyses are needed to confirm these previous results. Smith et al.³³ evaluated 350 genes in particular including genes involved

in pain treatment, as *TAARI*, *RGS4*, *CNRI*, and *GRIA4*. In fact, impaired *TAAR*-mediated dopamine availability could enhance pain sensitivity, a typical symptom in FM subjects.³⁴ *RGS4* gene, expressed in the locus coeruleus, the bed nuclei of the stria terminalis, and in the dorsal horn of the spinal cord,³⁵ plays a modulatory role in the descending inhibition of pain perception. *CNRI* encodes to CB-1 cannabinoid receptor, and its variants have been shown related with other pain diseases, like migraine,³⁶ irritable bowel syndrome,³⁷ and post-traumatic stress disorder.³⁸ *GRIA4* encodes the AMPA sensitive, ionotropic glutamate receptor subunit *GluR4*, which mediates fast excitatory transmission of nociceptive signals in the CNS and it is presumably involved in the central sensitization.³⁹ These studies improved the knowledge about FM and supported the genetic hypothesis underlying its pathogenesis, suggesting potential genetic markers for FM susceptibility, even though universally validated SNPs have not yet been found. Potential explanations are the population specificity of genetic variants and, moreover, being FM a multifactorial condition, haplotypes, combinations of different variants, might affect the improved risk of FM development more than a single variant: A correlation of the disease and the “high pain sensitivity” haplotype (*ACCG*) belonged to *COMT* gene in a Spanish population⁴⁰ and the *B2-AR AC* haplotype in Mexican and Spanish populations were already identified.⁴¹

Environmental influences on the occurrence of FM

Beside a genetic predisposition to FM, environment may be involved in the development of the disease. In particular, early-life events, including both physical trauma and psychosocial stressors have been found to influence gene expression and thus contribute to the occurrence of FM.^{42,43}

The evidence that physical trauma influence FM development in adulthood results from studies where the impact of early life pain experiences was evaluated: Early and childhood experiences have been associated with long-lasting changes in nociceptive circuitry and increases pain sensitivity in the older organism.⁴⁴ For example, adverse events during the neonatal and childhood life, like premature birth,⁴⁵ physical and sexual abuse,^{46,47} have been shown to possibly contribute to an alteration of threshold pain in adulthood and the development of FM onset.⁴⁸ As result of stress events, an impairment of HPA (hypothalamic-pituitary-adrenal) axis could rise up, with a subsequent inefficient response to stress and enhanced sensitivity to pain and fatigue.⁴⁹

In adulthood, repeated physical stressors have been demonstrated to be involved in the development of chronic widespread pain, particularly due to activities

like heavy lifting, repetitive motions, or squatting for extended periods of time.⁵⁰

Among the researches on environmental triggers of FM, psychological and social stressors seem to represent the strong predictors of the disease, including chronic stress, emotional trauma,⁵¹ with physical assault/abuse in women particularly associated with FM diagnoses.⁵² Other environmental conditions recently discovered to affect FM are childhood maltreatment, as neglect, emotional abuse, and post-traumatic stress disorder. Interestingly, concomitant levels of depression and anxiety were significantly higher among these FM patients.⁵³ A bidirectional temporal association between depression and FM has also been demonstrated, with an increase risk to develop each other.⁵⁴ In support of this connection, altered gray and white matter morphometry including medial orbitofrontal cortex and cerebellum have been observed in FM patients, with the gray matter volume associated with the severity of depression and hyperalgesia.⁵⁵ This finding suggests a potential shared pathophysiological mechanism underlying FM and depression.

Stressful life events in FM patients persist even in spite of different cultures, demonstrating the transcultural soundness of the association between diagnoses of FM in adulthood, self-reported childhood maltreatments, and lifelong traumatic experiences.⁵⁶

The physiological processes mediating the connection between experienced stress and the development of FM are still unknown.⁵⁷ The HPA axis failure has been proposed as potential responsible of this relationship,^{58,59} the increased pain levels of FM patients, in fact, have been found related to decreased levels of hypothalamic corticotrophin-releasing hormone⁵⁸ and an increased levels of substance P and glutamate in cerebrospinal fluid (CSF).⁵⁷ Moreover, hypoactivity of dopaminergic, opioidergic, and serotonergic systems have been evidenced in patients with FM, suggesting a complex derangement of psychobiological patterns.³⁹

Based on this evidence, environmental factors, particularly chronic stress and traumatic experiences, can be hypothesized to influence neurophysiological responses through gene expression alteration, in turn interfering with peripheral and central pain perception.

Recent studies suggest that also environment and HPA axis reactions to stress have a great impact on gut microbial composition and balance, which in turn affect human brain health, auto-immune reactions, and encephalotoxic metabolites release. The correlation between host genetics and microbiome has already been explored in pathologies as diabetes and obesity.⁶⁰ Concerning FM, the observed mitochondrial dysfunction,⁶¹ associated to pain sensitization and muscle pain, has been recently hypothesized to be potentially caused by a

gastrointestinal microbial imbalance, revealing new possible research lines for FM understanding and treatment.⁶²

The role of epigenetics: A new point of view

Previous studies demonstrated that early life experience and environmental factors in general could modulate genome function and the phenotype through epigenetic mechanisms, without altering the DNA sequence.⁶³ Main epigenetic mechanisms, supporting gene-environment interaction, are DNA methylation, covalent histone modifications, and non-coding RNAs. Epigenetic mechanisms have been observed to play an important role as mediators of long-term changes in central and peripheral nervous systems in chronic pain.⁶⁴ The environmental components observed in FM pathogenesis highlight a possible role of the gene-environment interaction in the development of this condition.

In particular, changes in methylation state, histone modifications, and miRNAs expression in pain-related regions appear to occur in the presence of peripheral inflammation and nerve injury.^{65–67} Being chronic pain one of the main symptoms of FM, knowledge about how pain-related genes and environment interact may shed light on the etiological mechanism underlying this condition.

Studies on DNA methylation and FM

DNA methylation biochemical process involves the addition of a methyl group to the fifth carbon of DNA cytosine residues, leading to 5-methylcytosines. The process occurs mainly in cytosines and guanines rich regions, CpG islands, located in the 60% of human gene promoters,⁶⁸ and is mediated by a group of DNA methyltransferases (DNMTs): DNMT1, DNMT3a, and DNMT3b.⁶⁹

A genome-wide DNA methylation study on healthy female monozygotic and dizygotic twins proved the implication of DNA methylation in thermal pain sensitivity.⁷⁰ In particular, a strong correlation of DNA methylation level in the promoter of *TRPA1* gene, expressed in peripheral nociceptors, and gate pain-related responses was identified.^{71,72} Higher levels of *TRPA1* expression was related to lower DNA methylation state in its promoter and higher pain thresholds. A consistent link between level of DNA methylation state and heat pain sensitivity in healthy subjects was demonstrated.⁷² DNA methylation alterations in FM patients have been also recently revealed^{73,74} (Table 2).

The first study investigating epigenetic changes in FM women compared to controls was a genome-wide methylation pattern analysis that highlights 69 differentially methylated sites in cases against controls, and 91% of these sites were responsible of an increased micronuclei

Table 2. Genes differentially methylated in FM women.

Gene	Biological samples	Physiological function	Associations
<i>BDNF</i> ⁷³	Blood	Neuron Differentiation/nervous system development	Mood disorders Alzheimer Parkinson Huntington's disease
<i>NAT15</i> ⁷³	Blood	Histone acetyltransferase Chromatin compaction	Acetylation process Facilitation of transcription process
<i>HDAC4</i> ⁷³	Blood	Deacetylation of the core histones Muscle maturation	Deacetylation's process Gene silencing
<i>PRKCA</i> ⁷³	Blood	Cell signaling pathways	Post-traumatic stress syndrome Emotional memory formation Cancer
<i>RTN1</i> ⁷³	Blood	Secretion or membrane trafficking in neuroendocrine cells	Neurological diseases Cancer
<i>PRKG1</i> ⁷³	Blood	Regulation cardiovascular and neuronal functions Relax smooth muscle tone Prevent platelet aggregation Modulate cell growth	Aortic aneurysm Phosphoglycerate kinase deficiency
<i>SLC17A9</i> ⁷⁴	Blood	Regulation neuronal differentiation	Neuronal plasticity
<i>TFAP2A</i> ⁷⁴	Blood	Survival functions of sympathetic progenitors and noradrenergic neurons	Neuronal circuits

A general hypomethylated pattern in FM patients compared to healthy subjects seem to be revealed, considering the first studies on DNA methylation and FM.

frequency in FM women.⁷³ This correlation should be further investigate as useful tool evaluation and/or diagnosis. Genes mapped on differently methylated sites were *BDNF*, *NAT15*, *HDAC4*, *PRKCA*, *RTN1*, and *PRKG1*, suggesting the possible involvement of nervous system development, skeletal/organ system development, and chromatin compaction pathways in FM. More recently, Ciampi de Andrade et al.⁷⁴ have investigated DNA methylation state in blood samples from a cohort of 24 FM cases and 24 healthy controls. The results identified 1610 differentially methylated positions: 1042 (65%) were found hypomethylated and 568 (35%) hypermethylated in cases compared to controls. Most of the differentially methylated genes were related to signal transduction and calcium signaling, MAPK signaling pathway, regulation of actin cytoskeleton endocytosis, and neuroactive ligand-receptor interaction pathways.⁷⁴ In general, the differentially methylated sites identified associated with FM map on genes involved in biological processes as DNA repair, immune system, and membrane transport genes. The mechanisms behind FM may thus include pathways related to autonomic system response, subcortical neuronal abnormalities, and impaired cellular response to stress and to glutathione,⁷⁴ potentially explaining the significantly deregulated oxidative and antioxidative parameters observed in FM women.⁷⁵ However, these changes may not be specific to FM but due to concurrent conditions.

Cortical excitability parameters were also measured in both hemispheres of FM cases and controls, they

resulted altered in parallel with methylation level changes in peripheral blood of FM patients.⁷⁴ This finding reveal the importance of DNA methylation research in peripheral blood to potentially develop biological markers of FM in the future.

MicroRNA profiles as new potential biomarkers

MicroRNAs are short non-coding RNA molecules approximately 20 to 22 nucleotides in length, highly evolutionary conserved; these factors have a fundamental role in the regulation of gene expression in disease processes and physiological pathways, since they are involved in cell growth, differentiation, stress response, and tissue remodeling; they exert several regulatory functions as mRNA cleavage, translational repression, or mRNAs deadenylation within cells where they were initially transcribed.⁷⁶ MicroRNAs regulate at least 30% of human genes,⁷⁷ and each microRNAs can repress hundreds of genes.⁷⁸ The presence of microRNAs in different cellular compartments and their stability in extracellular environment⁷⁹ make them attractive candidate biomarkers to better understand the etiology of complex disease like FM (Table 3).

They can be packaged with argonaute proteins or be transposed into biological fluids through exosomes. A fundamental role of miRNAs was observed in chronic pain conditions,⁸⁰ in which they alter and modulate the expression of signaling molecules, transmitters, ion channels, or structural proteins, contributing to develop

Table 3. MiRNAs differentially expressed in FM women compared with healthy controls.

miRNAs	Regulation in FM	Biological sample	Clinical symptoms
miR-145-5p ⁸²	Down	CSF	Pain and fatigue
miR-21-5p ⁸²	Down	CSF	Alteration of central circuits
miR-195-5p ⁸²	Down	CSF	Alteration in energy metabolism and growth Dementia
miR-223-3p ⁸²	Down	CSF	Inflammatory pain
miR-23a-3p ⁸²	Down	CSF	No correlation found
miR-23b ⁸²	Down	CSF	Alteration of μ -opioid receptor expression Alteration of outcome to long-term morphine treatment
miR-320a ⁸²	Up	Serum	Pain threshold
miR-107	Down	Serum	No correlation found
miR-151a-5p			
miR-142-3p ⁸⁶			
miR-30b-5p ⁸⁶	Down	Serum	Sleep quantity
miR-374b-5p ⁸⁶	Down	Serum	Pain threshold
miR-103a-3p	Down	Serum	Sleep quantity
let-7a-5p ⁸⁶			Pain
miR-451a	Down	PBMCs	No correlation found
miR-338-3p			
miR-143-3p			
miR-145-5p			
miR-223-3p ⁸⁹			
miR-23a-3p ⁹⁰	Down	Serum	Maintenance of skeletal muscle integrity
miR-1	Down	Serum	No correlation found
miR-133a		Saliva	
miR-346			
miR-139-5p			
miR-320b ⁹⁰			

Some miRNAs (highlighted) are equally deregulated across different tissue like **miR223-3p** and **miRNA-145-5p** that have been found to be inhibited in both PBMCs and CSF of FM patients, and **miR-23a-3p** that has been found downregulated in both serum and CSF. CSF: cerebro spinal fluid; FM: fibromyalgia; PBMC: peripheral blood mononuclear cells.

long-term hyperexcitability in nociceptive neurons in the periphery and CNS.⁸¹ A microRNAs genome-wide expression profile in FM women CSF, collected at rest by lumbar puncture through the L3/4 interspace, was assessed by Bjersing et al.;⁸² the relation with peculiar FM symptoms including pain threshold,⁸³ levels of pain,⁸⁴ and fatigue⁸⁵ was also explored. The study was conducted on 10 women with FM compared to 8 age-matched healthy controls. Nine out of 742 human miRNAs total assayed were significantly differently expressed in CSF between FM and healthy controls;⁸² the interaction with pain and fatigue was subsequently examined, and only **miR-145-5p** showed a significant correlation in FM patients. The same authors analyzed also circulating miRNAs in the serum of 20 FM patients⁸⁶ matched with healthy controls, identifying a different pattern from CSF micro-RNAs in FM.⁸² Eight out of 374 total human miRNAs analyzed were differentially expressed: **miR-320a** expression was higher in FM patients than healthy controls, while the expression of the remaining seven microRNAs (**miR-103a-3p**, **miR-107**, **let-7a-5p**, **miR-30b-5p**, **miR-151a-5p**, **miR-142-3p**,

and **miR-374b-5p**) was lower in FM cases compared to healthy subjects. Concerning the interaction with FM symptoms, **miR-30b-5p** correlated with sleep quantity in FM patients and **miR-374b-5p** was found inversely correlated with pain threshold; also **let-7a-5p** and **miR-103a-3p** tended to be associated with sleep quantity and pain. Lastly, **miR-320a**, higher expressed in FM, was inversely correlated with pain. These results seem to indicate a specificity of these processes in the periphery compared to the CNS: More researches should investigate this point since the study was conducted on restricted portion of the miRNAs sequenced available.^{87,88} MiRNAs regulating genes related to the immune system have been potentially assumed involved in FM onset; to this purpose, miRNAs expression using 1212 probes in peripheral blood mononuclear cells were examined, and decreased expression of specific miRNAs was revealed.⁸⁹ In particular, 5 miRNAs, **miR-451a**, **miR-338-3p**, **miR-143-3p**, **miR-145-5p**, and **miR-223-3p**, showed a 6- to 13-fold inhibition in FM patients compared to controls. Even if no correlation with clinical criteria was found, **miR223-3p** and

miRNA-145-5p might be proposed as biomarkers of the disease since they were also found to be inhibited in CSF of FM patients.⁸²

More recently, Masotti et al.⁹⁰ conducted a study on accurately selected FM patients, excluding drugs' use and thus avoiding variations of miRNA expression arising from analgesics.⁹¹ The expression of six miRNAs has proved to be downregulated (*miR-23a-3p*, *miR-1*, *miR-133a*, *miR-346*, *miR-139-5p*, and *miR-320b*) in FM patients compared to controls and, interestingly, *miR-23a* was downregulated in both CSF⁸² and serum of FM patients, although not significantly associated with FM symptoms.⁸²

Interestingly, *mir-23a* is implicated in a cluster with *miR27a/24-2*, responsible of *MURF1* and *MAFbx* down-regulation, two genes encoding ubiquitin ligases specific for muscle atrophy.⁹² This evidence suggests a potential involvement of this miRNA in the maintenance of skeletal muscle integrity.⁹³ In general, miRNAs found dysregulated in FM patients appear to be involved in physical activity, pain, stress, mood disorders, and depressive symptoms; therefore, a good predictive model with high diagnostic power should probably include many of these traits-associated miRNAs. Further studies need to strengthen these preliminary findings in larger cohorts.

Histone modifications

Histone modifications are covalent post-translational modifications of histone proteins' N-terminal tails (H1, H2A, H2B, H3 e H4), in particular methylation, phosphorylation, acetylation, ubiquitylation, and sumoylation.⁹⁴ They alter chromatin structure and subsequently affect different biological processes, as DNA repair process,⁹⁵ gene transcription and translation,⁹⁶ and ageing process.⁹⁷ One of the most studied histone modifications in pain is acetylation/deacetylation, the addition or removal of acetyl groups on N-terminal lysine residues and on nucleosome surface. Acetylation mechanism, operated by histone acetyltransferase enzymes, mediates the shift from condensed to relaxed chromatin, more accessible to transcriptions factors; conversely, deacetylation, made by histone deacetylases (*HDACs*), closely condenses chromatin resulting in gene silencing.⁹⁸ *HDAC* inhibitors in pain conditions emerged to be potentially implicated in analgesia, in both inflammatory and neuropathic pain.^{99,100} Their clinical effect is thought to be partially attributed to the reduced production of inflammatory cytokines such as *TNF- α* and *IL-1*.¹⁰¹ However, histone modifications in FM patients have not yet been investigated.

Gene expression

Since epigenetic mechanisms modulate gene expression, studies investigated transcription changes comparing FM patients and controls: FM alterations in gene expression should be viewed considering that they might not be exclusively related to FM pathology because of FM concomitant diagnoses, as osteoarthritis, depression, and obesity.¹⁰²

A recent analysis identified 482 differentially expressed genes between patients and healthy controls, shedding light on the relationship between FM status and upregulated inflammatory cytokines' genes (*IL10*, *IL25*, and *IL36A*).¹⁰² *IL-10*, one of the most powerful anti-inflammatory cytokines,¹⁰³ has been shown to regulate substance P expression, thus probably increasing the pain threshold. *IL-25*¹⁰⁴ was found to upregulate the expression of pro-inflammatory cytokines, especially Th2 cytokines. Both these cytokines have been proposed as key mediators of Th2 cytokine response, linked to chronic fatigue syndrome. In addition, several solute carrier molecules' genes were found upregulated in FM subjects including *SLC1A5* and *SLC25A22*, which encode for glutamate transporters in the CNS.¹⁰⁵ The metabotropic glutamate receptor gene (*GRM6*), encoding for a group III G protein-coupled receptor linked to the inhibition of the cyclic AMP cascade and involved in neuropathic pain signaling in dorsal horn neurons, was also upregulated in FM subjects.¹⁰⁶

A dysregulation of these pathways^{105,106} may be relevant to the pathogenesis of FM and thus need to be validated in a large, multicenter, independent cohort of subjects with greater clinical heterogeneity. In addition, no studies investigated if epigenetic mechanisms reflect the observed changes in gene expression.

Conclusions

FM is a complex disorder characterized by chronic pain, joint stiffness, fatigue, sleep disturbance, cognitive dysfunction, and depression. Research on FM is becoming increasingly important because of patients impaired quality of life and for the economic burden placed on the medical care system. FM patients often show concomitant diagnoses, such as osteoarthritis, depression, and obesity, with the consequently high risk of misdiagnosis. Most of the studies have been thus focused on research of specific and measurable biomarkers to objectively identifying susceptible individuals, to confirm disease diagnosis, and to facilitate treatment.

To achieve these goals, many familial studies were conducted demonstrating an increased risk to develop FM in first-degree relatives; candidate gene studies highlighted potential mechanisms involved in FM pathogenesis, identifying associated SNPs to the disease:

central sensitization to pain and HPA axis impairment. Beside a genetic predisposition, environmental factors, like infant trauma, stress, and depression, play a fundamental role in the onset and development of FM, through epigenetic modulations. In particular, a hypomethylation state is shown in FM patients compare to healthy controls, especially in promoter of genes implicate in DNA repair, immune system, and membrane transport genes. Many studies investigated miRNAs expression in FM condition in a variety of biological samples, highlighting the involvement of both peripheral and central processes.

It should be noted that many of the epigenetics studies have been performed on blood samples. Despite DNA methylation patterns are tissue specific¹⁰⁷ and their study in chronic pain should be thus limited to the brain. Recently, a correspondence across different tissues emerged: Massart et al.¹⁰⁸ have found that 72% of the genes affected in T cells were also differentially methylated in prefrontal cortex post-nerve injury; other studies have identified a correspondence between 35% and 80% of known transcripts in both peripheral blood and brain tissues.¹⁰⁹ The observed correspondences identify blood samples as a reliable and more accessible source of FM biomarkers. This paper reviewed relevant FM studies in order to better understand the still unclear mechanisms underlying this complex disease. However, some of the results should be considered with caution in light of the following limitations, representing also important directions for future researches. Despite the relatively high prevalence of FM, many studies included small size sample and, because of FM comorbidities, enrolled patients with no precise exclusion criteria and attention for ongoing therapies. In addition, to clarify the temporal onset between FM and its additional symptoms, the use of longitudinal follow-ups could be considered. Details on patients' history could improve everyday clinical practice: Dietary and lifestyle that may lead to alteration in gastrointestinal microbiome, observed in this complex disease, should be also deepened.

If the obtained preliminary data will be confirmed, research in future could focus on the identification of more selective analgesics or new pharmacological approaches, as currently being testing against pain of diverse etiologies, including Postherpetic Neuralgia,¹¹⁰ Inherited Erythromelalgia,¹¹¹ and Lumbosacral Radiculopathy,¹¹² with three phase II clinical trials on selective blockers drugs for sodium channels Nav1.7. In particular, since epigenetics plays a major role in regulating expression of pro- or antinociceptive genes, epigenetic drugs might potentially reverse aberrant gene expression profiles associated with FM states. First, pre-clinical data suggest chromatin-modifying drugs relevance for treating pain in particular in the context of inflammation¹¹³: 5-azacytidine administration in rats

following tissue damage resulted in the inhibition of global DNA methylation increment and MeCP2 expression with subsequent decrease of painful behavior.¹¹⁴

Drugs targeting epigenetic mediators as histone deacetylase and acetylase or involving DNA methylation maintenance have been developed for different pathological conditions.¹¹⁵ Similarly, improving FM biomarkers research, a new treatment scenario based on personalized medicine may be revealed, with major benefits and less side effects for FM patients and reduced cost for the national health-care systems.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

ORCID iD

Simona D'Agnelli  <http://orcid.org/0000-0001-5396-9542>

References

1. Jones GT, Atzeni F, Beasley M, FluB E, Sarzi-Puttini P and Macfarlane GJ. The prevalence of fibromyalgia in the general population: a comparison of the American College of Rheumatology 1990, 2010, and modified 2010 classification criteria. *Arthritis Rheumatol* 2015; 67: 568–575.
2. Choi DH and Kim HS. Quantitative analysis of nailfold capillary morphology in patients with fibromyalgia. *Korean J Intern Med* 2015; 30: 531–537.
3. Son CN, Kim SH, Chang HW and Kim JM. A neuro-metabolite study of chronic daily headache in patients with systemic lupus erythematosus using magnetic resonance spectroscopy: comparison with fibromyalgia patients and healthy controls. *Korean J Intern Med* 2016; 31: 1171–1177.
4. Clauw DJ. Fibromyalgia: a clinical review. *JAMA* 2014; 311: 1547–1555.
5. Prados G, Miró E, Martínez MP, Sánchez AI, López S and Sáez G. Fibromyalgia: gender differences and sleep-disordered breathing. *Clin Exp Rheumatol* 2013; 31: S102–S110.
6. Wolfe F, Ross K, Anderson J and Russell IJ. Aspects of fibromyalgia in the general population: sex, pain threshold, and fibromyalgia symptoms. *J Rheumatol* 1995; 22: 151–156.
7. Bradley LA. Pathophysiology of fibromyalgia. *Am J Med* 2009; 122: S22.
8. Branco JC. State-of-the-art on fibromyalgia mechanism. *Acta Reumatol Port* 2010; 35: 10–15.
9. Baek SH, Seok HY, Koo YS and Kim BJ. Lengthened cutaneous silent period in fibromyalgia suggesting central

- sensitization as a pathogenesis. *PLoS One* 2016; 11: e0149248.
10. Kosek E and Hansson P. Modulatory influence on somatosensory perception from vibration and heterotopic noxious conditioning stimulation (HNCS) in fibromyalgia patients and healthy subjects. *Pain* 1997; 70: 41–51.
 11. Julien N, Goffaux P, Arsenault P and Marchand S. Widespread pain in fibromyalgia is related to a deficit of endogenous pain inhibition. *Pain* 2005; 114: 295–302.
 12. Sluka KA. Neurobiology of fibromyalgia and chronic widespread pain. *Neuroscience* 2016; 338: 114–129.
 13. O'Brien AT, Deitos A and Triñanes Pego Y. Defective endogenous pain modulation in fibromyalgia: a meta-analysis of temporal summation and conditioned pain modulation paradigms. *J Pain* 2018; 19: 819–836.
 14. Millan MJ. Descending control of pain. *Prog Neurobiol* 2002; 66: 355–474.
 15. Gutierrez T, Nackley AG, Neely MH, Freeman KG, Edwards GL and Hohmann AG. Effects of neurotoxic destruction of descending noradrenergic pathways on cannabinoid antinociception in models of acute and tonic nociception. *Brain Res* 2003; 987: 176–185.
 16. Loggia LM, Berna C, Kim J, Cahalan CM, Gollub RL, Wasan AD, Harris RE, Edwards RE, and Napadow V. Disrupted brain circuitry for pain-related reward/punishment in fibromyalgia. *Arthritis Rheumatol* 2014; 66: 203–212.
 17. Gracely RH, Petzke F, Wolf JM, and Clauw DJ. Functional magnetic resonance imaging evidence of augmented pain processing in fibromyalgia. *Arthritis Rheum* 2002; 46: 1333–1343.
 18. Wolfe F, Smythe HA, Yunus MB, and Bennett RM. The American College of Rheumatology 1990 criteria for the classification of fibromyalgia. Report of the multicenter criteria committee. *Arthritis Rheum* 1990; 33: 160–172.
 19. Wolfe F, Clauw DJ, Fitzcharles MA, Goldenberg DL, Katz RS, Mease P, Russell AS, Russell IJ, Winfield JB, and Yunus MB. The American College of Rheumatology preliminary diagnostic criteria for fibromyalgia and measurement of symptom severity. *Arthritis Care Res* 2010; 62: 600–610.
 20. Wolfe F, Clauw DJ, Fitzcharles MA, Goldenberg DL, Häuser W, Katz RL, Mease PJ, Russell AS, Russell IJ, and Walitt B. 2016 Revisions to the 2010/2011 fibromyalgia diagnostic criteria. *Semin Arthritis Rheum* 2016; 46: 319–329.
 21. Ablin JN, Buskila D. Update on the genetics of the fibromyalgia syndrome. *Best Pract Res Clin Rheumatol* 2015; 29: 20–28.
 22. Mogil JS. Pain genetics: past, present and future. *Trends Genet* 2012; 28: 258–266.
 23. Oertel B and Lotsch J. Genetic mutations that prevent pain: implications for future pain medication. *Pharmacogenomics* 2008; 9: 179–194.
 24. Arnold LM, Fan J, Russell IJ, Yunus MB, Khan MA, Kushner I, Olson JM, and Iyengar SK. The fibromyalgia family study: a genome-wide linkage scan study. *Arthritis Rheum* 2013; 65:1122–1128.
 25. Mergener M, Becker RM, dos Santos AF, dos Santos GA, and de Andrade FM. Influence of the interaction between environmental quality and T102C SNP in the HTR2A gene on fibromyalgia susceptibility. *Rev Bras Reumatol* 2011; 51: 594–602.
 26. Cohen H, Buskila D, Neumann L and Ebstein RP. Confirmation of an association between fibromyalgia and serotonin transporter promoter region (5-HTTLPR) polymorphism, and relationship to anxiety-related personality traits. *Arthritis Rheum* 2002; 46: 845–847.
 27. Mickle AD, Shepherd AJ and Mohapatra DP. Sensory TRP channels: the key transducers of nociception and pain. *Prog Mol Biol Transl Sci* 2015; 131: 73–118.
 28. Fernández-de-Las-Peñas C, Ambite-Quesada S, Gil-Crujera A, Gil-Crujera A, Cigarán-Méndez M, and Peñacoba-Puente C. Catechol-O-methyltransferase Val158Met polymorphism influences anxiety, depression, and disability, but not pressure pain sensitivity, in women with fibromyalgia syndrome. *J Pain* 2012; 13: 1068–1074.
 29. Vargas-Alarcón G, Fragoso JM, Cruz-Robles D, Vargas A, Martínez A, Lao-Villadóniga JJ, García-Fructuoso F, Vallejo M, and Martínez-Lavín M. Association of adrenergic receptor gene polymorphisms with different fibromyalgia syndrome domains. *Arthritis Rheum* 2009; 60: 2169–2173.
 30. Gürsoy S, Erdal E, Herken H, Madenci E, and Alaşehirli B. Association of T102C polymorphism of the 5-HT2A receptor gene with psychiatric status in fibromyalgia syndrome. *Rheumatol Int* 2001; 21: 58–61.
 31. Lee YH, Choi SJ, Ji JD, and Song GG. Candidate gene studies of fibromyalgia: a systematic review and meta-analysis. *Rheumatol Int* 2012; 32: 417–426.
 32. Docampo E, Escaramis G, Gratacos M, Villatoro S, Puig A, Kogevinas M, Collado A, Carbonell J, Rivera J, Vidal J, Alegre J, Estivill X, and Rabionet R. Genome-wide analysis of single nucleotide polymorphisms and copy number variants in fibromyalgia suggest a role for the central nervous system. *Pain* 2014; 155: 1102–1109.
 33. Smith SB, Maixner DW, Fillingim RB, Slade G, Gracely RH, Ambrose K, Zaykin DV, Hyde C, John S, Tan K, Maixner W, and Diatchenko L. Large candidate gene association study reveals genetic risk factors and therapeutic targets for fibromyalgia. *Arthritis Rheum* 2012; 64: 584–593.
 34. Wood PB, Schweinhardt P, Jaeger E, Dagher A, Hakyemez H, Rabiner EA, Bushnell MC, and Chizh BA. Fibromyalgia patients show an abnormal dopamine response to pain. *Eur J Neurosci* 2007; 25: 3576–3582.
 35. Gold SJ, Ni YG, Dohlman HG and Nestler EJ. Regulators of G-protein signaling (RGS) proteins: region-specific expression of nine subtypes in rat brain. *J Neurosci* 1997; 17: 8024–8037.
 36. Juhasz G, Lazary J, Chase D, Pegg E, Downey D, Toth ZG, Stones K, Platt H, Mekli K, Payton A, Anderson IM, Deakin JF, and Bagdy G. Variations in the cannabinoid receptor 1 gene predispose to migraine. *Neurosci Lett* 2009; 461: 116–120.

37. Park JM, Choi M-G, Cho YK, Lee IS, Kim SW, Choi KY, and Chung IS. Cannabinoid receptor 1 gene polymorphism and irritable bowel syndrome in the Korean population: a hypothesis-generating study. *J Clin Gastroenterol* 2011; 45: 45–49.
38. Lu AT, Ogdie MN, Järvelin M-R, Moilanen IK, Loo SK, McCracken JT, McGough JJ, Yang MH, Peltonen L, Nelson SF, Cantor RM, and Smalley SL. Association of the cannabinoid receptor gene (CNR1) with ADHD and post-traumatic stress disorder. *Am J Med Genet B Neuropsychiatr Genet* 2008; 147B: 1488–1494.
39. Bleakman D, Alt A, Nisenbaum ES. Glutamate receptors and pain. *Sem Cell Dev Biol Pain* 2006; 17: 592–604.
40. Vargas-Alarcón G, Fragoso JM, Cruz-Robles D, Vargas A, Vargas A, Lao-Villadóniga JI, García-Fructuoso F, Ramos-Kuri M, Hernández F, Springall R, Bojalil R, Vallejo M, and Martínez-Lavín M. Catechol-O-methyltransferase gene haplotypes in Mexican and Spanish patients with fibromyalgia. *Arthritis Res Ther* 2007; 9: R110.
41. Vargas-Alarcón G, Fragoso JM, Cruz-Robles D, Vargas A, Martínez A, Lao-Villadóniga JI, García-Fructuoso F, Vallejo M, and Martínez-Lavín M. Association of adrenergic receptor gene polymorphisms with different fibromyalgia syndrome domains. *Arthritis Rheum* 2009; 60: 2169–2173.
42. Al-Allaf AW, Dunbar KL, Hallum NS, Nosratzadeh B, Templeton KD, and Pullar T. A case-control study examining the role of physical trauma in the onset of fibromyalgia syndrome. *Rheumatology* 2002; 41: 450–453.
43. Gur A, Kaakoc M, Nas K, Remzi, Cevik, Denli A, and Saraç J. Cytokines and depression in cases with fibromyalgia. *J Rheumatol* 2002; 29: 358–361.
44. Low LA and Schweinhardt P. Early life adversity as a risk factor for fibromyalgia in later life. *Pain Res Treat* 2012; 2012: 140832.
45. Klingmann PO, Kugler I, Steffke TS, Bellingrath S, Kudielka BM, and Hellhammer DH. Sex-specific prenatal programming: a risk for fibromyalgia? *Ann N Y Acad Sci* 2008; 1148: 446–455.
46. Paras ML, Murad MH, Chen LP, Goranson EN, Sattler AL, Colbenson KM, Elamin MB, Seime RJ, Prokop LJ, and Zirakzadeh A. Sexual abuse and lifetime diagnosis of somatic disorders: a systematic review and meta-analysis. *JAMA* 2009; 302: 550–561.
47. Häuser W, Kosseva M, Üceyler N, Klose P, and Sommer C. Emotional, physical, and sexual abuse in fibromyalgia syndrome: a systematic review with meta-analysis. *Arthritis Care Res* 2011; 63: 808–820.
48. Low LA and Schweinhardt P. Early life adversity as a risk factor for fibromyalgia in later life. *Pain Res Treat* 2012; 2012: 140832.
49. Tikkanen R, Ducci F, Goldman D, Holli M, Lindberg N, Tiihonen J, and Virkkunen M. MAOA alters the effects of heavy drinking and childhood physical abuse on risk for severe impulsive acts of violence among alcoholic violent offenders. *Alcohol Clin Exp Res* 2010; 34: 853–860.
50. Harkness EF, Macfarlane GJ, Nahit E, Silman AJ, and McBeth J. Mechanical injury and psychosocial factors in the work place predict the onset of widespread body pain: a two-year prospective study among cohorts of newly employed workers. *Arthritis Rheum* 2004; 50: 1655–1664.
51. Bennett RM, Jones J, Turk DC, Russell IJ, and Matallana L. An internet survey of 2,596 people with fibromyalgia. *BMC Musculoskelet Disord* 2007; 8: 27.
52. Haviland MG, Morton KR, Oda K, and Fraser GE. Traumatic experiences, major life stressors, and self-reporting a physician-given fibromyalgia diagnosis. *Psychiatry Res* 2010; 177: 335–341.
53. Hellou R, Häuser W, Brenner I, Buskila D, Jacob G, Elkayam O, Aloush V, and Ablin JN. Self-reported childhood maltreatment and traumatic events among Israeli patients suffering from fibromyalgia and rheumatoid arthritis. *Pain Res Manag* 2017; 2017: 3865249.
54. Chang MH, Hsu JW, Huang KL, Su TP, Bai YM, Li CT, Yang AC, Chang WH, Chen TJ, Tsai SJ, and Chen MH. Bidirectional association between depression and fibromyalgia syndrome: a nationwide longitudinal study. *J Pain* 2015; 16: 895–902.
55. Kim H, Kim J, Loggia ML, Cahalan C, Garcia RG, Vangel MG, Wasan AD, Edwards RR, and Napadow V. Fibromyalgia is characterized by altered frontal and cerebellar structural covariance brain networks. *Neuroimage Clin* 2015; 7: 667–777.
56. Häuser W, Hoffmann EM, Wolfe F, Worthing AB, Stahl N, Rothenberg R, and Walitt B. Self-reported childhood maltreatment, lifelong traumatic events and mental disorders in fibromyalgia syndrome: a comparison of US and German outpatients. *Clin Exp Rheumatol* 2015; 33: S86–S92.
57. Becker S and Schweinhardt P. Dysfunctional neurotransmitter systems in fibromyalgia, their role in central stress circuitry and pharmacological actions on these systems. *Pain Res Treat* 2012; 2012: 741746.
58. Crofford LJ, Pillemer SR, Kalogeras KT, Cash JM, Michelson D, Kling MA, Sternberg EM, Gold PW, Chrousos GP, and Wilder RL. Hypothalamic-pituitary-adrenal axis perturbations in patients with fibromyalgia. *Arthritis Rheum* 1994; 37: 1583–1592.
59. McCain GA and Tilbe KS. Diurnal hormone variation in fibromyalgia syndrome: a comparison with rheumatoid arthritis. *J Rheumatol Suppl* 1989; 19: 154–157.
60. Ussar S, Fujisaka S and Kahn CR. Interactions between host genetics and gut microbiome in diabetes and metabolic syndrome. *Mol Metab* 2016; 5: 795–803.
61. Cordero MD, Cano-García FJ, Alcocer-Gómez E, De Miguel M, and Sánchez-Alcázar JA. Oxidative stress correlates with headache symptoms in fibromyalgia: coenzyme Q10 effect on clinical improvement. *PLoS One* 2012; 7: e35677.
62. Vasquez A. Neuroinflammation in fibromyalgia and CRPS is multifactorial. *Nat Rev Rheumatol* 2016; 12: 242.
63. Szyf M and Bick J. DNA methylation: a mechanism for embedding early life experiences in the genome. *Child Dev* 2013; 84: 49–57.

64. Denk F and McMahon SB. Chronic pain: emerging evidence for the involvement of epigenetics. *Neuron* 2012; 73: 435–444.
65. Wang F, Stefano GB and Kream RM. Epigenetic modification of DRG neuronal gene expression subsequent to nerve injury: etiological contribution to complex regional pain syndromes (Part II). *Med Sci Monit* 2014; 20: 1188–1200.
66. Rahn EJ, Guzman-Karlsson MC and David SJ. Cellular, molecular, and epigenetic mechanisms in non-associative conditioning: implications for pain and memory. *Neurobiol Learn Mem* 2013; 105: 133–150.
67. Seo S, Grzenda A, Lomber G, Ou XM, Cruciani RA, and Urrutia R. Epigenetics: a promising paradigm for better understanding and managing pain. *J Pain* 2013; 14: 549–557.
68. Robertson KD. DNA methylation and human disease. *Nat Rev Genet* 2005; 6: 597–610.
69. Bestor TH. The DNA methyltransferases of mammals. *Hum Mol Genet* 2000; 9: 2395–2402.
70. Bell JT, Loomis AK, Butcher LM, Gao F, Zhang B, Hyde CL, Sun J, Wu H, Ward K, Harris J, Scollen S, Davies MN, Schalkwyk LC, Mill J; MuTHER Consortium, Williams FM, Li N, Deloukas P, Beck S, McMahon SB, Wang J, John SL, and Spector TD. Differential methylation of the TRPA1 promoter in pain sensitivity. *Nat Commun* 2014; 5: 2978.
71. Fajardo O, Meseguer V, Belmonte C and Viana F. TRPA1 channels mediate cold temperature sensing in mammalian vagal sensory neurons: pharmacological and genetic evidence. *J Neurosci* 2008; 28: 7863–7875.
72. Kwan KY, Allchorne AJ, Vollrath MA, Christensen AP, Zhang DS, Woolf CJ, and Corey DP. TRPA1 contributes to cold, mechanical, and chemical nociception but is not essential for hair-cell transduction. *Neuron* 2006; 50: 277–289.
73. Menzies V, Lyon DE, Archer KJ, Zhou Q, Brumelle J, Jones KH, Gao G, York TP, and Jackson-Cook C. Epigenetic alterations and an increased frequency of micronuclei in women with fibromyalgia. *Nurs Res Pract* 2013; 2013: 795784.
74. Ciampi de Andrade D, Maschietto M, Galhardoni R, Gouveia G, Chile T, Victorino Krepschi AC, Dale CS, Brunoni AR, Parravano DC, Cueva Moscoso AS, Raicher I, Kazyama HHS, Teixeira MJ, and Brentani HP. Epigenetics insights into chronic pain: DNA hypomethylation in fibromyalgia—a controlled pilot-study. *Pain* 2017; 158: 1473–1480.
75. Fatima G, Das SK and Mahdi AA. Some oxidative and antioxidative parameters and their relationship with clinical symptoms in women with fibromyalgia syndrome. *Int J Rheum Dis* 2017; 20: 39–45.
76. Filipowicz W, Bhattacharyya SN and Sonenberg N. Mechanisms of post-transcriptional regulation by microRNAs: are the answers insight? *Nat Rev Genet* 2008; 9: 102–114.
77. Lewis BP, Burge CB and Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell* 2005; 120: 15–20.
78. Baek D, Villén J, Shin C, Camargo FD, Gygi SP, and Bartel DP. The impact of microRNAs on protein output. *Nature* 2008; 455: 64–71.
79. Rayner KJ and Hennessy EJ. Extracellular communication via microRNA: lipid particles have a new message. *J Lipid Res* 2013; 54: 1174–1181.
80. Andersen HH, Duroux M and Gazerani P. MicroRNA as modulators and biomarkers of inflammatory and neuropathic pain conditions. *Neurobiol Dis* 2014; 71: 159–168.
81. Bartel DP. MicroRNAs: genomic, biogenesis, mechanism, and function. *Cell* 2004; 116: 281–297.
82. Bjersing JL, Lundborg C, Bokarewa MI and Mannerkorpi K. Profile of cerebrospinal microRNAs in fibromyalgia. *PLoS One* 2013; 8: e78762.
83. Kosek E, Ekholm J and Nordemar R. A comparison of pressure pain thresholds in different tissues and body regions. Long-term reliability of pressure algometry in healthy volunteers. *Scand J Rehabil Med* 1993; 25: 117–124.
84. Burckhardt CS, Clark SR and Bennett RM. The fibromyalgia impact questionnaire: development and validation. *J Rheumatol* 1991; 18: 728–733.
85. Ericsson A and Mannerkorpi K. Assessment of fatigue in patients with fibromyalgia and chronic widespread pain. Reliability and validity of the Swedish version of the MFI-20. *Disabil Rehabil* 2007; 29: 1665–1670.
86. Bjersing JL, Bokarewa MI and Mannerkorpi K. Profile of circulating microRNAs in fibromyalgia and their relation to symptom severity: an exploratory study. *Rheumatol Int* 2015; 35: 635–642.
87. Kozomara A and Griffiths-Jones S. miRBase: annotating high confidence microRNAs using deep sequencing data. *Nucleic Acids Res* 2014; 42: D68–D73.
88. Griffiths-Jones S. The microRNA registry. *Nucleic Acids Res* 2004; 32: D109–D111.
89. Cerdá-Olmedo G, Mena-Durán AV, Monsalve V, and Oltra E. Identification of a MicroRNA signature for the diagnosis of fibromyalgia. *PLoS One* 2015; 10: e0121903.
90. Masotti A, Baldassarre B, Guzzo MP, Iannuccelli C, Barbato C, and Di Franco M. Circulating microRNA profiles as liquid biopsies for the characterization and diagnosis of fibromyalgia syndrome. *Mol Neurobiol* 2017; 54: 7129–7136.
91. de Boer HC, van Solingen C, Prins J, Duijs JM, Huisman MV, Rabelink TJ, and van Zonneveld AJ. Aspirin treatment hampers the use of plasma microRNA-126 as a biomarker for the progression of vascular disease. *Eur Heart J* 2013; 34: 3451–3457.
92. Chhabra R, Dubey R and Saini N. Cooperative and individualistic functions of the microRNAs in the miR-23a~27a~24-2 cluster and its implication in human diseases. *Mol Cancer* 2010; 9: 232.
93. Wada S, Kato Y, Sawada S, Aizawa K, Park JH, Russell AP, Ushida T, and Akimoto T. MicroRNA-23a has minimal effect on endurance exercise-induced adaptation of mouse skeletal muscle. *Pflugers Arch* 2015; 467: 389–398.
94. Ricketts MD, Han J, Szurgot M and Marmorstein R. Molecular basis for chromatin assembly and modification

- by multi-protein complexes. *Protein Sci.* Epub ahead of print 23 October 2018. DOI: 10.1002/pro.3535.
95. Unal E, Arbel-Eden A, Sattler U, Shroff R, Lichten M, Haber JE, and Koshland D. DNA damage response pathway uses histone modification to assemble a double-strand break-specific cohesin domain. *Mol Cell* 2004; 16: 991.
 96. Karlič R, Chung HR, Lasserre J, Vlahoviček K, and Vingron M. Histone modification levels are predictive for gene expression. *Proc Natl Acad Sci* 2010; 107: 2926–2931.
 97. Fraga MF and Esteller M. Epigenetics and aging: the targets and the marks. *Trends Genet* 2007; 23: 413–418.
 98. Kuo MH and Allis CD. Roles of histone acetyltransferases and deacetylases in gene regulation. *Bioessays* 1998; 20: 615–626.
 99. Chiechio S, Zammataro M and Morales ME. Epigenetic modulation of mGlu2 receptors by histone deacetylase inhibitors in the treatment of inflammatory pain. *Mol Pharmacol* 2009; 75: 1014–1020.
 100. Chung YL, Lee MY, Wang AJ, and Yao LF. A therapeutic strategy uses histone deacetylase inhibitors to modulate the expression of genes involved in the pathogenesis of rheumatoid arthritis. *Mol Ther* 2003; 8: 707–717.
 101. Leoni F, Zaliani A, Bertolini G, Porro G, Pagani P, Pozzi P, Donà G, Fossati G, Sozzani S, Azam T, Bufler P, Fantuzzi G, Goncharov I, Kim SH, Pomerantz BJ, Reznikov LL, Siegmund B, Dinarello CA, and Mascagni P. The antitumor histone deacetylase inhibitor suberoylanilide hydroxamic acid exhibits antiinflammatory properties via suppression of cytokines. *Proc Natl Acad Sci U S A* 2002; 99: 2995–3000.
 102. Jones KD, Gelbart T, Whisenant TC, Waalen J, Mondala TS, Iklé DN, Salomon DR, Bennett RM, and Kurian SM. Genome-wide expression profiling in the peripheral blood of patients with fibromyalgia. *Clin Exp Rheumatol* 2016; 34: 89–98.
 103. O'Connor K, Sabat R, Friedrich M, Volk HD, Sterry W. Interleukin-10: an important immunoregulatory cytokine with major impact on psoriasis. *Curr Drug Targets Inflamm Allergy* 2004; 3:185–192.
 104. O'Connor TM, O'Connell J, O'Brien DI, Goode T, Bredin CP, and Shanahan F. The role of substance P in inflammatory disease. *J Cell Physiol* 2004; 201: 167–180.
 105. Nakamura T, Schwander Sk, Donnelly R, Ortega F, Togo F, Broderick G, Yamamoto Y, Cherniack NS, Rapoport D, and Natelson BH. Cytokines across the night in chronic fatigue syndrome with and without fibromyalgia. *Clin Vaccine Immunol* 2010; 17: 582–587.
 106. Montana MC and Gereau RW. Metabotropic glutamate receptors as targets for analgesia: antagonism, activation, and allosteric modulation. *Curr Pharm Biotechnol* 2011; 12: 1681–1688.
 107. Razin A and Szyf M. DNA methylation patterns. Formation and function. *Biochim Biophys Acta* 1984; 782: 331–342.
 108. Massart R, Dymov S, Millemcamps M, Suderman M, Gregoire S, Koenigs K, Alvarado S, Tajerian M, Stone LS, and Szyf M. Overlapping signatures of chronic pain in the DNA methylation landscape of prefrontal cortex and peripheral T cells. *Nature* 2016; 6: 19615.
 109. Tylee DS, Kawaguchi DM and Glatt SJ. On the outside, looking in: A review and evaluation of the comparability of blood and brain “-omes”. *Am J Med Genet* 2013; 162B: 595–603.
 110. Xenon Pharmaceuticals Inc. A phase 2a, randomized, double-blind, placebo-controlled, two-period crossover study to evaluate the safety, tolerability, preliminary efficacy, and systemic exposure of topical XPF-002 in subjects with postherpetic neuralgia, <https://ichgcp.net/clinical-trials-registry/NCT01195636>
 111. Xenon Pharmaceuticals Inc. Phase 2a, exploratory, double-blind, placebo-controlled two-part study to evaluate the safety, efficacy, tolerability and pharmacokinetics of topically applied XPF-002 (XEN402 8% w/w Ointment) in patients with primary/inherited erythromelalgia, <http://clinicaltrials.gov/ct2/show/NCT01486446?term=xen402&rank=2>
 112. Biogen. A randomized, double blind, cross-over study to evaluate the safety and efficacy of CNV1014802 in subjects with neuropathic pain from lumbosacral radiculopathy, <http://clinicaltrials.gov/ct2/show/NCT01561027?term=CNV1014802&rank=2>
 113. Crow M, Denk F and McMahon SB. Genes and epigenetic processes as prospective pain targets. *Genome Med* 2013; 5: 12.
 114. Wang Y, Liu C, Guo QL, Yan JQ, Zhu XY, Huang CS, and Zou WY. Intrathecal 5-azacytidine inhibits global DNA methylation and methyl-CpG-binding protein 2 expression and alleviates neuropathic pain in rats following chronic constriction injury. *Brain Res* 2011; 1418: 64–69.
 115. Szyf M. Epigenetics, DNA methylation, and chromatin modifying drugs. *Annu Rev Pharmacol Toxicol* 2009; 49: 243–263.